PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 13 August 2001 (13.08.01)	
International application No. PCT/GB00/03806	**Applicant's or agent's file reference** 9.32.73775/001
International filing date (day/month/year) 04 October 2000 (04.10.00)	**Priority date** (day/month/year) 04 October 1999 (04.10.99)
Applicant BROSENS, Jan	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

02 May 2001 (02.05.01)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Juan Cruz
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
9.32.73775/001		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 00/03806	04/10/2000	04/10/1999
Applicant		
BROSENS, Jan		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 1

   [X] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

12

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
9.32.73775/001		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB00/03806	04/10/2000	04/10/1999
International Patent Classification (IPC) or national classification and IPC C12N15/64		
Applicant BROSENS, Jan		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
02/05/2001	17.01.2002
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Petri, B Telephone No. +49 89 2399 7356

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB00/03806

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-35 as originally filed

Claims, No.:

1-23 as originally filed

Drawings, sheets:

1/5-5/5 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	
	No:	Claims	1-23
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-23
Industrial applicability (IA)	Yes:	Claims	1-23
	No:	Claims	

2. Citations and explanations
see separate sheet

The present application relates to the use of cell type specific differential cis-splicing patterns in cell type specific gene targeting.

Reference is made to the following document/s/:

D1: GUO W. & HELFMAN D.M.: 'Cis-elements involved in alternative splicing in the rat beta-tropomyosin gene: the 3'-splice site of the skeletal muscle exon 7 is the major site of blockage in nonmuscle cells' NUCL. ACIDS RES., vol. 21, no. 20, 1993, pages 4762-4768, XP002156466

D1 discloses a **genetic construct** comprising **splice functional el ments,** causing a particular part of the construct which sequence is not interrupted by stop codons (= open reading frame available for translation) to be present or absent from the expression product depending on the cell where the construct is expressed". (Abstract; Figs 1, 2, 5).

D2: WO 96 04391 A (GENENTECH INC) 15 February 1996 (1996-02-15)

D2 discloses a bicistronic expression vector wherein a selectable gene is flanked by splice functional elements. Depending on the efficiency of the splice functional elements the ratio of spliced vs. nonspliced = selectable gene absent or present is altered. As such there are different cell populations (=target cell or non target cell) in which the splice site is used or non used. (see e.g Examples 1-3). Consequently D2 discloses a **gen tic construct** comprising **splice functional elements,** causing a particular part of the construct which sequence is not interrupted by stop codons (= open reading frame available for translation) to be present or absent from the expression product depending on the cell where the construct is expressed".

D3: WO 97 22250 A (INTRONN LLC ;MITCHELL LLOYD G (US)) 26 June 1997 (1997-06-26)

D3 concerns the use of a transsplicing mechanism to selectively express target genes in selected target cell populations (see e.g. page 9 line 25 - page 10 line 21), and genetic constructs therefor.

Consequently D3 discloses a **genetic construct** comprising **splice functional elements,** causing a particular part of the construct which sequence is not interrupted by stop codons (= open reading frame available for translation) to be present or absent from the expression product depending on the cell where the construct is expressed" (see e.g. Figs. 1-2, Claims 1-4).

D4: COTE G.J. ET AL.: 'Sequence requirements for regulated RNA splicing of the human Fibroblast Growth Factor Receptor-1 alpha exon.' J. BIOL. CHEM., vol. 272, no. 2, 10 January 1997 (1997-01-10), pages 1054-1060, XP002156467 cited in the application

D4 is concerned with the analysis of the mechanisms for cell type specific exon skipping. The study uses **genetic constructs** comprising **splice functional elements,** causing a particular part of the construct which sequence is not interrupted by stop codons (= open reading frame available for translation) to be present or absent from the expression product depending on the cell where the construct is expressed" (see inter alia Fig. 6).

D6: KAWAMOTO S.: 'Neuron-specific alternative splicing of nonmuscle Myosin II Heavy Chain-B pre-mRNA requires a cis-acting intron sequence.' J. BIOL. CHEM., vol. 271, no. 30, 26 July 1996 (1996-07-26), pages 17613-17616, XP002156469 & GUO N.H. & KAWAMOTO S.: 'An intronic downstream enhancer promotes 3' splice site usage of a neural cell-specific exon.' J. BIOL. CHEM., vol. 275, no. 43, 27 October 2000 (2000-10-27), pages 33641-33649,

D6 concerns the study of the intron sequence responsible for neuron-specific splicing of MHC-B and genetic constructs therefore (see e.g. Figs. 2-3).

Consequently D6 discloses a **genetic construct** comprising **splice functional elements,** causing a particular part of the construct which sequence is not interrupted by stop codons (= open reading frame available for translation) to be present or absent from the expression product

depending on the cell where the construct is expressed".

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statem nt

Novelty; Art 33(2), PCT

The subject-matter proposed in claim 1-23 of the present application cannot be considered as novel (Article 33(2) PCT) for the following reasons.

The particular constructs as disclosed in the present application are new. The claims in their present form however lack nevertheless novelty in view of D1-D4, D6.

The technical features extractable from the definition of claim 1 are as following:

" A **genetic construct** comprising **splice functional elements,** causing a particular part of the construct which sequence is not interrupted by stop codons (= open reading frame available for translation) to be present or absent from the expression product depending on the cell used for expression".

All 5 documents describe genetic constructs comprising splice functional elements and their effect on the expression (splicing or non-splicing, absence or presence) of sequences / exons / minigenes / ORFs of interest.

As no further technical features are recognizable in the definition of claim 1 and dependent claims, the technical features of claims 1-23 are not sufficient to distinguish the claimed molecules from those of the prior art. As however, the basic concept underlying the present application appears novel and inventive, it appears worthwhile to spend further effort in identifying true technical features suitable for a proper definition.

INTERNATIONAL SEARCH REPORT

Interr nal Application No
PC[redacted] 00/03806

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/64 C12N15/85 C12N5/10 A61K31/70 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GUO W. & HELFMAN D.M.: "Cis-elements involved in alternative splicing in the rat beta-tropomyosin gene: the 3'-splice site of the skeletal muscle exon 7 is the major site of blockage in nonmuscle cells" NUCL. ACIDS RES., vol. 21, no. 20, 1993, pages 4762-4768, XP002156466 the whole document	1-5, 7-21,23
Y	---	6,22
Y	WO 96 04391 A (GENENTECH INC) 15 February 1996 (1996-02-15) abstract figure 1 claim 1	6,22
	--- -/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 January 2001	25/01/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Galli, I

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Interr nal Application No
PC[redacted] 00/03806

C.(Continuation) DOCUMENTS CONSIDERED [redacted] BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	✓ WO 97 22250 A (INTRONN LLC ;MITCHELL LLOYD G (US)) 26 June 1997 (1997-06-26) abstract	3,4
A	✓ COTE G.J. ET AL.: "Sequence requirements for regulated RNA splicing of the human Fibroblast Growth Factor Receptor-1 alpha exon." J. BIOL. CHEM., vol. 272, no. 2, 10 January 1997 (1997-01-10), pages 1054-1060, XP002156467 cited in the application the whole document	8,9
A	✓ ICHIDA M. ET AL.: "MyoD is indispensable for muscle-specific alternative splicing in mouse mitochondrial ATP synthase gamma-subunit pre-mRNA." J. BIOL. CHEM., vol. 272, no. 14, 3 April 1998 (1998-04-03), pages 8492-8501, XP002156468 the whole document & ICHIDA M. ET AL.: "Differential Regulation of Exonic Regulatory Elements for Muscle-specific Alternative Splicing during Myogenesis and Cardiogenesis " J. BIOL. CHEM., vol. 275, no. 21, 26 May 2000 (2000-05-26), pages 15992-16001,	10
A	✓ KAWAMOTO S.: "Neuron-specific alternative splicing of nonmuscle Myosin II Heavy Chain-B pre-mRNA requires a cis-acting intron sequence." J. BIOL. CHEM., vol. 271, no. 30, 26 July 1996 (1996-07-26), pages 17613-17616, XP002156469 the whole document & GUO N.H. & KAWAMOTO S.: "An intronic downstream enhancer promotes 3' splice site usage of a neural cell-specific exon." J. BIOL. CHEM., vol. 275, no. 43, 27 October 2000 (2000-10-27), pages 33641-33649,	11

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

...rormation on patent family members

Interr nal Application No
PCT 00/03806

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9604391 A	15-02-1996	US 5561053 A	01-10-1996
		AU 704408 B	22-04-1999
		AU 3204595 A	04-03-1996
		CA 2195303 A	15-02-1996
		EP 0770136 A	02-05-1997
		JP 10503376 T	31-03-1998
WO 9722250 A	26-06-1997	US 6013487 A	11-01-2000
		AU 1329997 A	14-07-1997
		US 6083702 A	04-07-2000
		CA 2240494 A	26-06-1997
		EP 0883344 A	16-12-1998